Molecular epidemiology and antimicrobial resistance of *Salmonella* Typhimurium DT104 on Ontario swine farms

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Abstract

This study was conducted to examine antimicrobial resistances, plasmid profiles, and pulsed-field gel electrophoresis patterns of 80 *Salmonella* Typhimurium (including var. Copenhagen) DT104 strains (including DT104a and DT104b) recovered from pig and environmental fecal samples on 17 swine farms in Ontario. No resistance was observed to amoxicillin/clavulanic acid, apramycin, carbadox, cephalothin, ceftriaxone, ceftiofur, cefoxitin, ciprofloxacin, nalidixic acid, trimethoprim, and tobramycin. However, the isolates exhibited resistance against 4 to 10 antimicrobials with the most frequent resistance being to sulfonamides (Su), ampicillin (A), streptomycin (S), spectinomycin (Sp), chloramphenicol (C), tetracycline (T), and florfenicol (F). Thirteen distinct resistance patterns were determined but 88% of isolates shared the typical resistance pattern "ACSpSSuT." Twelve different plasmid profiles were observed; the 62 MDa virulence-associated plasmid was detected in 95% of the isolates. The 2.1 MDa plasmid was the second most frequent one, which was harbored by 65% isolates. The isolates were classified into 23 distinct genotypes by PFGE-*Spe*I + *Bln*I when difference in at least one fragment was defined as a distinct genotype. In total, 39 distinct "types" were observed when defining a "type" based on the combination of antimicrobial resistance, plasmid pattern, and PFGE-*Spe*I + *Bln*I for each isolate. The highest diversity was 0.96 (95% CI: 0.92, 0.96) for the "type" described above followed by 0.92 (95% CI: 0.88, 0.93) for PFGE-*Spe*I + *Bln*I. The diversity of DT104 isolates indicates there might be multiple sources for this microorganism on swine farms. This knowledge might be used to track these sources, as well as to study the extent of human salmonellosis attributed to pork compared to food products derived from other food-producing animals.

Résumé

La présente étude a été effectuée afin d'étudier les résistances antimicrobiennes, les profils plasmidiques et les patrons d'électrophorèse en champs pulsé (PFGE) de 80 isolats de Salmonella *Typhimurium (incluant var. Copenhagen) DT104 (incluant DT104a et DT104b) provenant d'échantillons fécaux de porc et d'environnement sur 17 fermes porcines en Ontario. Aucune résistance envers les antibiotiques suivants n'a été observée : amoxicilline/acide clavulanique, apramycine, carbadox, céphalothine, ceftriaxone, ceftiofur, cefoxitin, ciprofloxacin, acide nalidixique, triméthoprime et tobramycine. Toutefois, les isolats ont démontré de la résistance envers 4 à 10 antimicrobiens, avec les résistances les plus fréquentes étant dirigées vers : sulfonamides (Su), ampicilline (A), streptomycine (S), spectinomycine (Sp), chloramphénicol (C), tétracycline (T) et florfénicol (F). Treize patrons distincts de résistance ont été déterminés mais 88 % des isolats partageaient le patron de résistance typique «ACSpSSuT». Douze profils plasmidiques différents ont été observés; le plasmide de virulence de 62 MDa fut détecté chez 95 % des isolats. Un plasmide de 2,1 MDa était le deuxième plus fréquent, et était retrouvé chez 65 % des isolats. Les isolats ont été classés en 23 génotypes distincts par PFGE à l'aide des enzymes* SpeI + BlnI *lorsqu'une différence dans au moins un fragment était défini comme étant un génotype distinct. Au total, 39 «types» distincts ont été observés lorsqu'on définissait un «type» sur la base d'une combinaison, et ce pour chacun des isolats, de la résistance antimicrobienne, du patron plasmidique et du patron de PFGE-*SpeI + BlnI. *La plus grande diversité était de 0,96 (intervalle de confiance 95 % [CI] : 0,92, 0,96) pour le «type» décrit ci-dessus, suivi de 0,92 (CI 95 % : 0,88, 0,93) pour le PFGE-*SpeI + BlnI. *La diversité des isolats de DT104 indique qu'il pourrait y avoir des sources multiples de cet organisme sur les fermes porcines. Ces informations pourraient être utilisées pour retracer ces sources, ainsi que pour étudier l'ampleur des cas de salmonellose humaine attribués au porcs comparativement aux produits alimentaires dérivés des autres animaux fournissant des produits carnés.*

(Traduit par Docteur Serge Messier)

Introduction

Multi-drug resistant *Salmonella* Typhimurium DT104 was first isolated from a human case of salmonellosis in the UK as early as 1980 (1). Since then it has been isolated from humans and other sources including food-producing animals around the world; it has become a worldwide public health concern (2). *Salmonella* Typhimurium DT104 first demonstrated a typical pattern of

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penta-resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline (ACSSuT), but it has more recently displayed additional resistance to other antimicrobials. Multi-drug resistant *Salmonella* Typhimurium DT104 has also been the first or second most common *Salmonella* serovar reported from human and food-producing animals in Canada (3–6) and it has been found to be associated with increased hospitalization, mortality, and consequent economic cost (7–8).

As a non-host adapted *Salmonella* serovar, *Salmonella* Typhimurium DT104 has been isolated from different sources including poultry (9), swine (10–11) companion animals (12), cattle (13), horses (14), and food products (15–18). During the recent past, *Salmonella* Typhimurium DT104 has been the most frequently isolated in epidemiological studies in pork slaughterhouses (19), and from clinically ill pigs (20) in Canada. *Salmonella* Typhimurium var. Copenhagen DT104 has been reported as the second most common phage type on swine farms in Alberta (21). Multi-drug resistant *Salmonella.* Typhimurium DT104 has also been reported from other food-producing animals (4,6,17) in Canada; however, *Salmonella* Typhimurium DT104 strains isolated from different sources might not be distinguished based on phenotypic characteristics. For the purpose of designing control programs it is critical to understand how DT104 bacteria are introduced, transmitted, and maintained on farms, as well as to have knowledge of the source-specific attributable fraction for human salmonellosis. Therefore, molecular techniques that discriminate among *Salmonella* Typhimurium DT104 strains must be used to perform further epidemiological investigations.

Different molecular techniques including polymerase chain reaction (PCR) to identify unique gene sequences, amplified fragment length polymorphism (AFLP), ribotyping, pulsed-field gel electrophoresis (PFGE), and repetitive palindromic extragenic-PCR (Rep-PCR) have been used recently to investigate the molecular determinants and genetic relatedness between *Salmonella* Typhimurium DT104 isolates on pig farms (11,22–25). The molecular and antimicrobial resistance diversity among *Salmonella* Typhimurium DT104 strains isolated from different animal sources including cattle, poultry, and swine throughout Canada has been reported previously (26). Also diversity in antimicrobial resistance and genotypes of DT104 strains isolated from pigs in slaughterhouses has been studied recently (19). The objective of this study was to investigate the diversity in antimicrobial resistance and molecular characteristics of 80 *Salmonella* Typhimurium DT104 strains recovered from apparently healthy pigs on 17 swine farms in Ontario between 2001 and 2004.

Materials and methods

Bacterial isolates

All of the isolates in this study originated from another study that is described in more detail elsewhere (27). Briefly, a subset of 100 Ontario swine farms was tested for *Salmonella* by culturing fecal samples in 2001, 2003, and 2004. These farms had been selected initially for participation in a large surveillance study of Ontario pig farms. A portion of these 100 farms had originally been chosen using a stratified random sample based on herd size, as well as a portion that were purposively selected in order to have a balanced geographical representation. The sampling strategy was used to include farms in eastern Ontario and the Niagara region where there are relatively few pig farms. In 2003, 9 liquid-feeding farms were purposively added using a convenience sampling from the list of members of the Ontario Swine Liquid-feeding Association. In addition, a small number of farms were conveniently selected; generally farms close to Guelph Ontario. Similarly, a convenience sample of farms was added to the study population as replacements for operations which either stopped producing pigs or stopped participating in the study.

In total, all 80 *Salmonella* Typhimurium DT104 (including 74 *Salmonella* Typhimurium var. Copenhagen, 5 *Salmonella* Typhimurium, and 1 *Salmonella* I:4,12:i:-) that were recovered from pig fecal samples on 17 farms in 2001, 2003, and 2004 were included in the study. The isolates were phage type DT104 (42 isolates), DT104a (23 isolates), and DT104b (15 isolates). Three *Salmonella* Typhimurium DT104 isolated on 1 farm in 2001, 11 *Salmonella* Typhimurium var Copenhagen (5 DT104 and 6 DT104a isolates) recovered on 4 farms in 2003, and 66 strains (34 DT104, 17 DT104a, and 15 DT104b isolates) recovered from samples collected on 14 farms in 2004, were examined. Fifty of the 80 isolates were recovered from feces collected directly from pigs (pig sample) and 30 isolates were cultured from fresh fecal samples found on the floor of the pen (environmental sample).

Antimicrobial-susceptibility testing

Antimicrobial susceptibility of *Salmonella* isolates was tested by using the agar dilution method (28). Susceptibility breakpoint levels and the reference strains used were those described by the National Committee for Clinical Laboratory Standards (NCCLS) M100-S12 (29) (for most antimicrobials) and M31-A2 (30) (for apramycin, neomycin, spectinomycin, and streptomycin). Susceptibility to ceftiofur and carbadox were tested at breakpoint level as described in previous studies (31,32). Briefly, the isolates were cultured in Muller Hinton (MH) broth to obtain 0.5–1.0 McFarland density and using a Cathra Replicator plated onto MH agar plates containing antimicrobials (Sigma-Aldrich, St. Louis, Missouri, USA). The antimicrobials tested were; amikacin (Amk) at 16 μg/mL, ampicillin (A) at 32 μg/mL, amoxicillin and clavulanic acid (Ac) at 64 μg/mL and 16 μg/mL, respectively, apramycin (Apr) at 32 μg/mL, carbadox (Car) at 30 μg/mL, cephalothin (Ceph) at 32 μg/mL, ceftriaxone (Ceft) at 8 μg/mL, ceftiofur (Ceftif) at 8 μg/mL, cefoxitin (Cefox) at 32 μg/mL, chloramphenicol (C) at 32 μg/mL, ciprofloxacin (Cip) at 0.125 μg/mL, florfenicol (F) at 16 μg/mL, gentamicin (G) at 16 μg/mL, kanamycin (K) at 64 μg/mL, nalidixic acid (Nal) at 32 μg/mL, neomycin (N) at 16 μg/mL, nitrofurantoin (Nit) at 64 μg/mL, spectinomycin (Sp) at 64 μg/mL, streptomycin (S) at 32 μg/mL, sulfisoxazole (sulfonamides) (Su) at 512 μg/mL, tetracycline (T) at 16 μg/mL, tobramycin (Tob) at 8 μg/mL, and trimethoprim (Tm) at 16 μg/mL. To determine resistance to florfenicol, aquaflor (Schering Plough Animal Health, Pointe Claire, Quebec) containing 50% florfenicol was dissolved in dimethylformamide (28). After a 24 h incubation at 37°C, the plates were examined for bacterial growth and isolates that grew were considered to be resistant. The reference strains used were *Escherichia coli* ATCC 25922,

Table I. Antimicrobial resistance patterns among 80 *Salmonella* Typhimurium (including var. Copenhagen) isolates from pig feces and environment on 17 swine farms in Ontario

R-type	Resistance pattern	Number of isolates	Percent
1	ACFSpSSuT	45	56.2
2	ACFKNSpSSuT	21	26.2
3	ACGKNSpSSu	3	3.7
4	ACFKNSpSSu	2	3.7
5	ACFNitSpSSuT	2	2.5
6	ACFKNNitSpSSuT	1	1.2
7	ACFNitSpSSuT	1	1.2
8	AFKNNitSpSSuT	1	1.2
9	ACFSpSSu	1	1.2
10	SpSuSxtTm	1	1.2
11	ACFKNSSuT	1	1.2
12	ASSuT	1	1.2
Total		80	100

Escherichia coli ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853, as described in the NCCLS standards M100-S12 (29) and M31-A2 (30). A bovine strain R1022 possessing the aac(3)IV gene and resistant to apramycin, gentamicin, and tobramycin and other antimicrobials was also used.

Plasmid profiling

Plasmid finger printing was performed as explained by Poppe et al (28). Briefly, plasmid deoxyribonucleic acid (DNA) was extracted using the alkaline lysis method, then electrophoresed, and visualized by staining with ethidium bromide and subsequently exposing the ethidium bromide-DNA complexes to ultraviolet light (33). Plasmids used as the marker were: pSLT2 62 Mega Daltons (MDa), pDT285 (96 MDa), and pDT369 (23 MDa), and the 8 plasmids of *E. coli* V517 with a molecular weight of 1.4 to 35.8 MDa.

Pulsed-field gel electrophoresis (PFGE)

Pulsed-field gel electrophoresis (PFGE) was performed as previously described by the Centers for Disease Control and Prevention (CDC) (34). Briefly, agarose plugs containing whole DNA were prepared and slices were digested for 18 h with restriction enzymes, *Spe*I or *Bln*I. Whole-cell DNA of *Salmonella* Newport am01144 restricted with *Xba*I was used as a molecular size marker. The PFGE patterns were determined as described by Liebisch and Schwarz (35). Results were analyzed with BioNumerics (Applied Maths, Austin, Texas, USA) using the Dice similarity coefficient (optimization 1.5%, position tolerance 1.5%). The Dice similarity coefficient was calculated for each pair of isolates (I and II) using the following formula (36):

$$S_D = 2a \ / \ (2a + b + c) \quad (Equation\ 1)$$

where:

a is the number of bands present in both isolates, *b* represents the number of bands absent in isolate I but present in isolate II, and *c* is the number of bands present in I but absent in isolate II.

A similarity < 100% was assigned as 2 different genotypes in that a difference in at least 1 fragment was defined as a distinct genotype. Also the similarity coefficient was used to create the dendograms using the Unweighted Pair Group for Arithmetic Means (UPGMA). The similarity in the composite dendogram (*Bln*I and *Spe*I together) is calculated by taking the average from each of the individual analyses (*Bln*I and *Spe*I).

Diversity

Simpson's index was used to investigate diversity among the *Salmonella* Typhimurium DT104 isolates (37). The PAST software (38) was used to compute the Simpson's index (diversity index), which takes into account the total number of isolates, the number of groups created by each method, and the number of isolates in each group. The formula for the Simpson's index is:

$$1 - \sum (n_i / n)^2 \quad (Equation\ 2)$$

where:

n_i is the number of isolates in the i*th* group, and *n* is total number of isolates.

The 95% confidence interval (CI) for the Simpson's index was obtained by using a bootstrap procedure in PAST. The diversity among isolates recovered from pig samples was compared with the diversity among those isolated from environmental samples. In order to adjust for clustering, a logistic regression with pen and farm as the random variables and type of sample as the fixed effect was used to determine whether there was a difference in the isolates recovered from the pig and pen environments (39).

Results

Antimicrobial resistance

All isolates were susceptible to amoxicillin/clavulanic acid, apramycin, carbadox, cephalothin, ceftriaxone, ceftiofur, cefoxitin, ciprofloxacin, nalidixic acid, trimethoprim, and tobramycin. However, the isolates exhibited resistance against 4 to 10 antimicrobials with most frequent resistance to sulfonamides (100%), ampicillin (99%), streptomycin (99%), spectinomycin (97%), chloramphenicol (96%), tetracycline (93%), and florfenicol (93%). A lower level of resistance was observed to neomycin (39%) and kanamycin (38%), while only a small number of isolates demonstrated resistance to nitrofurantoin (6%) and gentamicin (4%), and these were exhibited only by isolates recovered from pig samples. Twelve distinct resistance patterns (R-type 1 to 12) (Table I) were determined with "ACFSpSSuT" and "ACFKNSpSSuT" as the 2 most frequent resistance patterns representing by 56% and 26% of the isolates, respectively. The typical DT104 R-type "ACSpSSuT," however, was present among 88% of isolates.

Except for resistance to tetracycline, which was exhibited by 100% of *Salmonella* Typhimurium DT104 isolates recovered from environmental samples compared to 88% of "pig samples" (*P* < 0.05), and resistance to gentamicin and nitrofurantoin, which was exhibited only by DT104 strains isolated from pig samples, there was no significant difference in antimicrobial resistance between DT104

Table II. Plasmid patterns among 80 *Salmonella* Typhimurium (including var. Copenhagen) DT104 isolates on 17 swine farms in Ontario

P-type	Plasmid pattern (MDa)	Number of isolates	Percent
a	62, 2.1	49	61.2
b	62	21	26.2
c	62, 3.0	3	3.7
d	4.8, 2.1	1	1.2
e	50, 40, 38	1	1.2
f	62, 2.8	1	1.2
g	62, 36, 2.1	1	1.2
h	62, 4.0, 2.1	1	1.2
I	65	1	1.2
j	65, 1.4	1	1.2
Total		80	100

Table III. Different types[a] found among 80 *Salmonella* Typhimurium (including var. Copenhagen) DT104 isolates on 17 swine farms in Ontario

Type	Number of isolates	Number of farms	Type	Number of isolates	Number of farms
1aD	9	4	12eP	1	1
1bH	6	2	13fU	1	1
2aM	6	2	1bD	1	1
1aE	5	3	1bE	1	1
2aH	5	1	1bJ	1	1
1bG	4	1	1dT	1	1
1aK	3	2	1gK	1	1
1bS	3	1	2aN	1	1
1aC	2	2	2aO	1	1
1bF	2	1	2aS	1	1
1bR	2	1	2aV	1	1
1dQ	2	1	3cM	1	1
2aA	2	1	5eW	1	1
2aD	2	2	5hT	1	1
2aI	2	1	6iT	1	1
3cL	2	1	6IS	1	1
4cM	2	1	7aE	1	1
10mB	1	1	8eT	1	1
11jS	1	1	9kM	1	1

[a] The definition of "type" was based on the combination of antimicrobial resistance (1 to 12), plasmid pattern (a to j), and PFGE-S*pe*I + *Bln*I (A to W) for each isolate.

isolated from pig and environmental samples. Resistance to kanamycin and neomycin, however, was significantly correlated to phage type in that 91% of DT104a displayed resistance to these 2 antimicrobials compared to 19% and 7% of DT104 and DT104b, respectively ($P < 0.0001$).

Plasmid profiling

Of the 12 plasmids detected, the 62 MDa plasmid was detected most frequently; it was harbored by 95% of the isolates either alone or in combination with other plasmids. This plasmid was observed in 88% of the isolates that were resistant to ampicillin, chloramphenicol, spectinomycin, streptomycin, sulfonamides, and tetracycline. The 2.1 MDa plasmid, which was harbored by 65% isolates, was the 2nd most frequent plasmid. Ninety-three percent of isolates resistant to kanamycin and neomycin had this plasmid. In fact, all susceptible kanamycin and neomycin isolates lacked the 2.1 MDa plasmid. Also all isolates resistant to gentamicin and nitrofurantoin contained both 62 MDa and 2.1 MDa plasmids. Overall, 10 different plasmid profiles (P-type: a to j) were determined with "62, 2.1," and "62" was the most frequent P-types carried by 61% and 26% of isolates, respectively (Table II).

Pulsed-field gel electrophoresis (PFGE)

The isolates were classified into 7 different genotypes when using *Spe*I (PFGE-*Spe*I) with 2 large groups containing 92% isolates. However, 18 genotypes were determined when restricting with *Bln*I (PFGE-*Bln*I) in which 51% of isolates belonged to 3 larger groups. In total, 23 genotypes were generated when analyzing digestion with both *Bln*I and *Spe*I (PFGE-*Spe*I + *Bln*I: A to W) with a Dice similarity index ranging between 35% and 100%. The 5 larger groups represented 58% of isolates. In total, the isolates recovered from pig samples in 18 pens on 10 different farms were discriminated from the isolates recovered from environmental samples from these same pens by PFGE-*Spe*I + *Bln*I. However, these isolates were identical based on phage type, antimicrobial resistance pattern, and plasmid profile. Only isolates recovered from pig and environmental samples from 2 pens on 2 different farms had identical PFGE patterns.

Isolate classification

A "type" was defined based on the combination of antimicrobial resistance pattern, plasmid profile, and PFGE-*Spe*I + *Bln*I for each isolate. For example, 1 isolate with R-type: 1, P-type: a, and PFGE-*Spe*I + *Bln*I: D was defined as type: 1aD. In total, 38 distinct types were identified of which 17 types contained 2 to 9 isolates and the remaining 21 types had only 1 isolate each (Table III). Three similar types were identified on 2 farms. In total, 5, 4, 3, 2, and 1 type (s) were distinguished on 4, 2, 4, 4, and 3 farms, respectively.

Diversity

The overall diversity of 80 isolates, and diversity among the isolates recovered from pig and environmental samples are shown in Table IV. The highest diversity was 0.96 (95% CI: 0.92, 0.96) when defining a type based on an antimicrobial resistance pattern, plasmid profile, and PFGE-*Spe*I + *Bln*I followed by 0.92 (95% CI: 0.88, 0.93) for PFGE-*Spe*I + *Bln*I while the lowest diversity among the isolates was seen for PFGE-*Spe*I and plasmid profiling. Except diversity in antimicrobial resistance, there was no significant difference in diversity among the isolates recovered from pig samples compared to those isolated from environmental samples.

Discussion

The objective of this study was to investigate the diversity in antimicrobial resistance pattern, plasmid profile, and PFGE pattern among the *Salmonella* Typhimurium DT104 isolates on 17 swine farms in Ontario. It was found that 88% of isolates shared the typical R-type "ACSSuT," which has been frequently reported in

Table IV. Diversity (Simpson's index) with 95% confidence interval among 80 *Salmonella* Typhimurium (including var. Copenhagen) DT104 isolates obtained by antimicrobial susceptibility testing, plasmid profiling, and PFGE

	Pig samples	Environment samples	Total
AMR	0.67 (0.52, 0.77)	0.91 (0.84, 0.91)	0.88 (0.84, 0.90)
Plasmid	0.56 (0.37, 0.68)	0.55 (0.40, 0.65)	0.55 (0.44, 0.62)
PFGE-*Spe*I	0.52 (0.39, 0.60)	0.34 (0.12, 0.51)	0.47 (0.34, 0.55)
PFGE-*Bln*I	0.85 (0.78, 0.88)	0.91 (0.84, 0.91)	0.88 (0.84, 0.90)
PFGE-(*Spe*I + *Bln*I)	0.91 (0.86, 0.92)	0.92 (0.84, 0.92)	0.92 (0.88, 0.93)
Type	0.95 (0.90, 0.95)	0.96 (0.92, 0.96)	0.96 (0.92, 0.96)

association with DT104 isolates from different sources in Canada (28) and other countries (25,40–42). Resistances to "ACT," "ACNT," and "ACNSSuTm" were the most common patterns that occurred among DT104 isolates recovered from healthy pigs in the slaughterhouses receiving animals from Quebec, Ontario, Manitoba, Saskatchewan, and British Columbia (19). In fact, the typical penta-resistance was not reported in that study at all. Particular resistance patterns might be related to certain animal species (22,28,43) and the discrepancy in resistance to additional antimicrobials might be useful to discriminate the DT104 isolates from different sources. The variation in antimicrobial resistance between isolates recovered on farm, at slaughter, and from samples submitted to diagnostic laboratories might represent some level of true diversity among DT104 isolates which might be used for investigating the specific source of salmonellosis and antimicrobial resistance in humans. The differences in antimicrobial resistance between different studies, however, might be partly due to between-laboratory variation. It should be noted that since we used the agar dilution test using a single breakpoint concentration of each antimicrobial, it is possible that there may have been some isolates that were classified as susceptible that would have been classified as resistant using an MIC method.

The number and size of plasmids might be used in combination with antimicrobial resistance to distinguish the isolates from different sources. However, the 62MDa plasmid, which was detected in almost 90% of ACSpSSuT-resistant isolates in this study, might not be associated with the DT104 isolates from a specific source (41) because it has been reported to be associated with penta-resistant DT104 isolates recovered from different sources in Canada (28) and other countries (25,44).

On the other hand, a lower diversity has been reported among the DT104 strains isolated from diseased pigs compared to the isolates shed by healthy carrier pigs (19). Since fecal samples were collected from "apparently" healthy pigs and from feces found on the pen floor, it was possible that some of *Salmonella* Typhimurium DT104 isolates from the environmental samples had been shed by clinical cases or were at least strains that were more likely to result in clinical illness. The use of different restriction enzymes to digest DNA, and the use of different criteria to define the relatedness (the difference in number of bands) in different studies, however, may have biased the comparisons.

Most isolates with typical penta-resistance were genotyped into different groups in the study. However, it has been shown that the DT104 strains with this typical penta-resistance pattern might have more than 90% similarity in PFGE and that the ACSSuT-resistant

DT104 isolated from swine and pork might be indistinguishable to those isolated from cattle and beef with PFGE-*Xba*I (41).

The isolates recovered from pig and pen environment samples could be classified into different genotypes by PFGE. A difference in at least 1 band was used to define a genotype; this approach has been used in other studies (19,45,46). However, this approach might have resulted in overestimation of the diversity among the DT104 isolates in this study. It is possible that due to being under different physical, chemical, and biological conditions, point mutations might have occurred among the isolates recovered from the environmental samples resulting in a 1-band difference on the gel. The difference in only 1 band, therefore, may not represent 2 distinct genotypes if the isolates were recovered from the same pen. The similarity between the isolates, however, ranged from 35% to 100% and some isolates differed in > 1 band indicating that DT104 isolates might have been introduced into swine farms from different sources. Nevertheless, if the genotype were to be defined as the difference in 5–7 bands, as suggested by Tenover et al (47) for outbreak investigation, there would then be only 1 identical clone of DT104 spreading on 17 Ontario swine farms despite the fact that the isolates belonged to 3 distinct phage types, 10 plasmid patterns, and 12 antimicrobial resistance patterns.

The different techniques demonstrated different degrees of diversity among the isolates in this study. However, 82% of isolates demonstrated the 2 predominant antimicrobial resistance patterns, 87% isolates had the 2 major plasmid profiles and 58% of the isolates belonged to the 5 larger PFGE groups. This may indicate that DT104 isolates in this study had a clonal distribution on swine farms but, to describe a clear diversity among DT104 isolates, definition of a "type" was based on the combination of antimicrobial resistance, plasmid pattern, and PFGE-*Spe*I + *Bln*I for each isolate. Using this approach, only 40% of isolates were classified into the 4 predominant "types." The classification of the isolates into different "types" may demonstrate the complexity of population structure of DT104 and should be interpreted with caution, particularly when comparing the isolates that were recovered from the same pen. The diversity among the "types" of DT104 isolates in this study, however, may indicate that DT104 isolates might be spread from different sources such as commingling pigs, rodents, insects, birds, and workers on the different farms; this is significant with respect to *Salmonella* control. There was also a difference in antimicrobial resistance, plasmid profiling, and PFGE genotypes among isolates recovered in 2001 and 2003 compared with those isolated in 2004. This may indicate that DT104 isolates have been introduced into the farms from different sources

at different times. Most isolates, however, were recovered in 2004 and this variation might result from inclusion of a smaller number of isolates from 2001 and 2003 compared with 2004. The changes in molecular characteristics of *Salmonella* Typhimurium DT104 on swine farms over time should be investigated in future studies.

In order to provide a more precise knowledge of DT104 on swine farms in Ontario, the antimicrobial resistance pattern, plasmid profile, and PFGE of each strain were combined and the "type" was defined for distinguishing the isolates. This knowledge might be used to track the source of DT104 on swine farms and to discover different sources by which the multi-resistant DT104 is introduced and maintained on swine farms. These DT104 genotypes can also be compared to those recovered from human cases to estimate the extent of human salmonellosis that may be attributed to pork.

Acknowledgments

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